poured into 20% HCl. The organic layer was separated, washed with water, and evaporated to dryness. The residue was steam distilled to remove naphthalene. The resulting gummy solid was dissolved in 400 ml. of petroleum ether containing 10% by volume of chloroform. The mixture was poured through a 30-cm. column packed with alumina, and eluted with the same solvent mixture. Forty-ml. fractions were collected and allowed to evaporate to dryness. Fractions 3–11 contained pale yellow crystalline material, while succeeding fractions were resinous and darker in color. The crystalline material from fractions 3–11 was combined and recrystallized from 3:1 isopropyl alcohol–ethyl acetate mixture, using charcoal. The yield was 0.40 g. (38%) of fine colorless needles, m.p. 252–256°. Three more recrystallizations from the same solvent mixture gave a product melting sharply at 270°. Attempts to purify crude tetra-1-naphthylgermane by crystallization without chromatographing gave only impure, low melting products. Tetra-1-naphthylgermane was sparingly soluble in alcohols but soluble in most other organic solvents.

Anal. Calcd. for $C_{40}H_{28}Ge$: Ge, 12.49. Found: Ge, 12.32.

This compound is relatively inert chemically. It was unreactive toward alcoholic KOH, bromine in CCl_4, $KMnO_4$, and dilute or concentrated mineral acids. It slowly dissolved in a warm mixture of sulfuric and fuming nitric acids in the course of the analysis.

Tri-1-naphthylgermane.—One gram of tri-1-naphthylgermanium bromide, in 20 ml. of ether, was added dropwise to 20 ml. of ether containing 0.2 g. of $LiAlH_4$. A white solid precipitated immediately. The mixture was heated under reflux for two hours, after which the excess hydride was decomposed by the dropwise addition of ethanol followed by water. The ethereal layer was separated, dried over $CaCl_2$, and evaporated to dryness. The residue was crystallized from benzene, yielding 0.7 g. of colorless crystalline tri-1-naphthylgermane, m.p. 240–246° (82%). Recrystallization from benzene and then from chloroform–petroleum ether gave colorless needles melting at 249–250°.

Anal. Calcd. for $C_{30}H_{22}Ge$: C, 79.17; H, 4.87. Found: C, 78.74; H, 5.05.

Tri-1-naphthylgermane showed little reaction with cold basic solutions; however, the compound gave a steady stream of hydrogen bubbles when warmed gently with KOH in moist piperidine.[3] Also, a CCl_4 solution of the germane rapidly decolorized bromine in the cold with the evolution of HBr.

Acknowledgment.—The author is grateful to Dr. Eugene G. Rochow for valuable encouragement and advice.

CAMBRIDGE, MASS.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE MALLINCKRODT CHEMICAL WORKS]

X-Ray Contrast Media. I. Iodinated Acylaminobenzoic Acids[1]

BY V. H. WALLINGFORD, HARRIET G. DECKER AND MARGARET KRUTY

RECEIVED APRIL 19, 1952

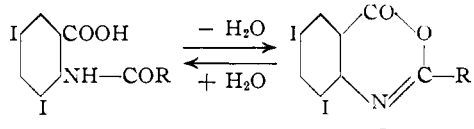
Sixteen new iodinated acylaminobenzoic acids have been prepared for investigation as X-ray contrast media. The introduction of a lower acyl group markedly lowers the toxicity of the parent iodinated aminobenzoic acid and increases the solubility of its sodium salt. From among the compounds reported, sodium 3-acetylamino-2,4,6-triiodobenzoate has been found to possess to a high degree the properties of low toxicity and high solubility desired for a urographic contrast medium. 2-Acylamino-3,5-diiodobenzoic acids are readily dehydrated to form cyclic anhydrides analogous to acetylanthranil. Analytical control procedures are given for preparing iodine monochloride reagent.

This is the first report of an investigation having for its objective the synthesis of organic compounds of possible use as X-ray contrast media for the visualization of various tissues, organs or cavities of the human body. Consideration of the aminobenzoic acids suggested a sufficient number of iodine-containing derivatives possibly to correlate structure to physical and pharmacological properties. Useful compounds have been found in the group of iodinated acylaminobenzoic acids reported in this paper.

The iodinated aminobenzoic acids or their derivatives had not found application in radiography previous to the present study. A Swiss patent[2] claims, as a useful medium, the monoethanolamine salt of 4-amino-3,5-diiodobenzoic acid. This compound (as the diethanolamine salt) was included in the present study for comparison. Its relatively high toxicity explains why it has not found acceptance. In 1944 Klemme and Bang[3] prepared azo dyes by coupling 4-amino-3,5-diiodobenzoic acid with naphthalene intermediates. Although it was stated that these compounds were to be tested as X-ray contrast media, no report has yet appeared.

The literature records several monoiodinated acetylaminobenzoic acids but these were not included in the present study because of their low iodine content. The only recorded acylaminobenzoic acid containing two or more iodine atoms is 2-acetylamino-3,5-diiodobenzoic acid described by Goldberg and his associates.[4]

The iodinated acylaminobenzoic acids, the properties of which are summarized in Table I, were prepared by the action of the appropriate acid anhydride or chloride upon the iodinated aminobenzoic acid with or without a diluent. The acid anhydrides usually require the presence of a trace of strong acid such as sulfuric acid for rapid reactions. The action of an excess of acetic anhydride upon 2-amino-3,5-diiodobenzoic acid produced a non-acidic compound apparently resulting from the loss of water from the acetylated amino-acid with the formation of an inner anhydride (I, R = CH_3)



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(1) Presented before the Division of Medicinal Chemistry, American Chemical Society, Milwaukee, March 31, 1952.

(2) Swiss Patent No. 175,169, July 16, 1935; *C. A.*, **30**, 246 (1936).

(3) C. J. Klemme and H. Bang, *J. Org. Chem.*, **9**, 254 (1944).

(4) A. A. Goldberg, H. S. Jefferies, H. S. Turner and D. M. Besly, *Quart. J. Pharm. Pharmacol.*, **19**, 483 (1946).

TABLE I

IODINATED AMINOBENZOIC ACIDS AND THEIR ACYL DERIVATIVES

Benzoic acid derivative	Procedure	Yield, %	M.p., °C.	Analysis,[e] % Calcd. C	Calcd. H	Found C	Found H	Solubility[m] Na salt g./100 cc.	LD_{50}, mg./kg.[r]
2-Amino-3,5-diiodo-	1	85.2[c]	242.5–243.5[d]	21.6	1.29	21.39	1.53	13.3	180
3-Amino-2,4,6-triiodo-	2	74.2[n]	196.5–197.5[d]	...	...	...	...	9.3	1450
4-Amino-3,5-diiodo-	3	78.8[n]	>310	...	...	...	...	2.56	515
2-Acetylamino-3,5-diiodo-	7	93.4[c]	228.5–230[q]	25.1	1.62	24.94	1.80	38.8	1530
2-Butyrylamino-3,5-diiodo-	7	85[n]	203.5–205	28.26	2.42	28.73	2.85	....	560
2-(*n*-Caproylamino)-3,5-diiodo-	6	90.5[c]	205.5–206.5[d]	32.0	3.08	32.44	3.85	105.0	365
2-Benzoylamino-3,5-diiodo-	6	90.0[n]	211.5–213[u]	34.08	1.84	34.08	2.04	...	360
2-(α-Phenylbutyrylamino)-3,5-diiodo-	6	84.0[n,s]	237.0–237.5[b]	38.13	2.83	38.45	2.75	....	385
2-(*o*-Iodobenzoylamino)-3,5-diiodo-	6[g]	94[n]	222.5–224.5[d]	27.15	1.30	26.78	1.75	....	575
3-Formylamino-2,4,6-triiodo-	8	71[n]	252[d]	I, 70.2		I, 69.0		70.5	8000
3-Acetylamino-2,4,6-triiodo-	4	92.5[n]	280–280.5[d]	19.4	1.08	19.68	1.50	94.2	9560[g]
3-Propionylamino-2,4,6-triiodo-	4[t]	92[c]	253–254[d]	I, 66.7		I, 66.9		37.2	8100
3-Butyrylamino-2,4,6-triiodo-	4[h]	66[n]	248[d]	22.57	1.72	22.65	2.01	59.3	4800
3-Benzoylamino-2,4,6-triiodo-	6[i]	87[c,i]	308[d]	27.15	1.30	27.29	1.55	15.9	1000
3-Isobutyrylamino-2,4,6-triiodo-	9	...	270–271[d]	22.58	1.72	22.73	1.99	29.3	3700
3-Caproylamino-2,4,6-triiodo-	9	65[n]	231–234	25.5	2.3	25.6	2.4	77.6	1450
				I, 62.1		I, 62.5			
3-Caprylylamino-2,4,6-triiodo-	9	38[n]	211.4–212.5	28.10	2.83	27.9	3.1	v.s.	660
3-Lauroylamino-2,4,6-triiodo-	9	34[n]	192–193	32.73	3.76	33.02	4.02	15.1	290
4-Acetylamino-3,5-diiodo-	4	96.3[c]	>310[k]	25.1	1.63	25.17	1.95	50.0	4200
4-Benzylamino-3,5-diiodo-	6[l]	39[c,l]	285–286	34.08	1.84	34.12	2.0	....	625

[a] Recrystallized from acetic acid. [b] Recrystallized from 50% dioxane and water. [c] Crude product. [d] With decomposition. [e] Average of two analyses. [f] Nitrogen, calcd. 2.62; found 2.86. [g] Refluxed 4 hr. Product precipitated at *p*H 5 leaving *o*-iodobenzoic acid in solution. [h] Equal volumes butyric anhydride and dioxane. Product precipitated very slowly from 15% alcohol solution by 3% HCl. [i] Refluxed 11 hr. during acylation. [j] Purified by precipitating the ammonium salt from 10% NH_4Cl solution. Neut. equiv., calcd. 431; found 428. [k] Recrystallized from 80% dioxane. The crude material before dissolving in alkali weighed more than the theoretical and melted about 230°. It probably contained some of the mixed anhydride. [l] The amino compound was heated with twice its weight of benzoyl chloride 5 hr. at 128–130° with no solvent. 65% recovery in purification. [m] Approximate solubilities in water at 25°. [n] Purified product. [q] Goldberg, *et al.*,[4] reported m.p. 214–216°. [r] LD_{50} intravenously in white mice, expressed as mg. of the free acid, determined by Lloyd W. Hazelton, Pharmacologist, Falls Church, Va. [s] Average of several determinations, varying slightly with concentration and rate of injection. [t] Heated 1 hr. at 125° for acylation. Purified by precipitating the ammonium salt from 10% NH_4Cl.

Compounds of Type I, the so-called acylanthranils, are well known, although no iodine containing member of the series has previously been prepared. The anhydro compounds are normal by-products of the acylation of 2-aminobenzoic acid, the excess acylating agent serving to dehydrate the open acyl compound first formed. The anhydroacyl derivatives in some cases were found to be convenient intermediates for preparing or purifying the desired open chain acyl compounds since they are readily converted to the latter type by hydrolysis. Having an anhydride-like structure, these compounds undergo interesting reactions which will be the subject of a subsequent publication.

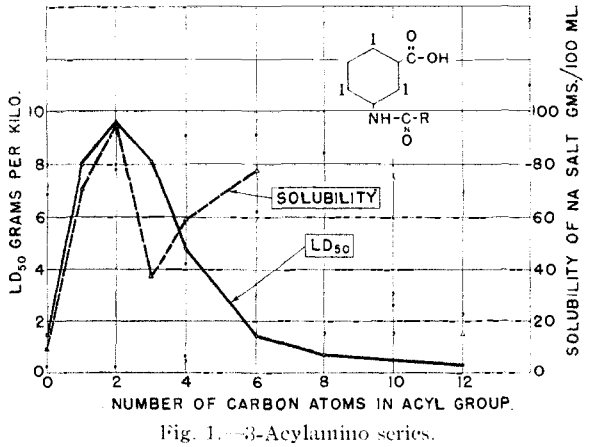

Fig. 1.—3-Acylamino series.

The data in Table I reveal some surprising and interesting trends. The introduction of a lower acyl group increases the solubility of the sodium salt and decreases the toxicity. In both the acylated and non-acylated series, the compounds derived from *m*-aminobenzoic acid are much less toxic than the corresponding ortho compounds, the para isomers having an intermediate toxicity.

In comparing the series of acyl derivatives of 3-amino-2,4,6-triiodobenzoic acid one finds a gradation in properties (Fig. 1). The introduction of the formyl group increases the LD_{50} from 1450 mg./kg. to 8000 mg./kg. The least toxic member of the series is the acetyl derivative with an LD_{50} of 9560 mg. kg. As the size of the substituting group is further increased the toxicity increases and the LD_{50} drops to 290 mg./kg. in the case of 3-lauroylamino-2,4,6-triiodobenzoic acid. The solubilities of the sodium salts follow a similar pattern for the lower members of the series. The higher members show a divergency, indicating that factors other than solubility in water are affecting toxicity.

The effect of branching of the chain is evident in one instance. 3-Isobutyryl-2,4,6-triiodobenzoic acid exhibits greater toxicity and lower solubility of its sodium salt than the compound containing the normal butyryl group.

Early attention was attracted to the sodium

salt of 3-acetylamino-2,4,6-triiodobenzoic acid because of its unusual properties. It is ten times as soluble and only one-sixth as toxic as the sodium salt of the parent 3-amino-2,4,6-triiodobenzoic acid. Its high iodine content (sodium salt, 65.8%; free acid, 68.4%) provides unusually high opacity to X-rays. Studies at the University of Michigan[5] showed that this compound is well tolerated intravenously and is rapidly eliminated by the kidneys in animals and in man. Clinical trials by Reed M. Nesbit and Jack Lapides[6] established sodium 3-acetylamino-2,4,6-triiodobenzoate as a useful new radiographic agent.[7]

It has been established[8] that replacement of the acetyl group in 3-acetylamino-2,4,6-triiodobenzoic acid by propionyl, butyryl or caproyl groups causes the compound to be excreted less by the kidneys of rats or dogs and to be collected to a progressively greater degree in the liver or gallbladder. No member of this series is significantly absorbed from the gastrointestinal tract of the dog. Further pharmacological studies are in progress and will be reported elsewhere.

The authors are indebted to Robert E. Taylor[9] for the preparation of 3-lauroylamino-2,4,6-triiodobenzoic acid, to Melvin A. Thorpe for arranging the pharmacological and clinical studies and to William R. Armstrong for assistance in preparing the manuscript.

Experimental

Typical procedures are numbered and are referred to in the table. All melting points are corrected.

Iodine Monochloride.—This reagent was prepared by direct addition of chlorine to iodine. The product was mixed with 5% of its weight of concentrated hydrochloric acid so that it would remain liquid at room temperature. The resulting mixture, containing approximately 95.2% ICl, was used for all iodination reactions. The following analytical methods were found useful when preparing the reagent.

Underchlorination was detected by dissolving 1–2 ml. (sp. gr. approx. 3.08) of the mixture in 25 ml. of concentrated hydrochloric acid, 25 ml. of water and 5 ml. of chloroform. An excess of iodine shows as a purple color in the chloroform layer after shaking. The amount of free iodine was determined by titration with potassium iodate solution (50 g. per liter) until the color in the chloroform disappeared. One ml. of iodate solution equals 0.1188 g. of iodine.

If no free iodine was present the mixture was examined for overchlorination by titrating with potassium iodide solution (50 g. per liter) until iodine color appeared in the chloroform layer. For each ml. of potassium iodide solution used, 0.0766 g. of iodine must be added to correct for overchlorination. Iodine monochloride concentration is conveniently determined by titrating a solution in dilute hydrochloric acid with thiosulfate using starch as an indicator. One ml. of 0.1 *N* thiosulfate equals 0.00812 g. of ICl. A correction must be made if excess iodine or iodine trichloride is present.

2-Amino-3,5-diiodobenzoic Acid (Procedure No. 1).—The earlier procedures of Wheeler and Johns[10] and Klemme and Hunter[11] were modified to give improved yields and a purer product. Anthranilic acid (68.5 g.) was dissolved in water (2500 ml.) and concentrated hydrochloric acid (50 ml.). To the slightly warm solution was added a mixture of iodine monochloride (185 g.) and concentrated hydrochloric acid (185 ml.). After the mixture had stood overnight at room temperature, the excess iodine monochloride was reduced by sodium sulfite (15 g.). The crude product (98% yield) was recrystallized as the ammonium salt from twenty times its own weight of ammonium chloride solution (1 part to 5 parts water). The ammonium salt was filtered off and washed with 20% ammonium chloride solution, and then was dissolved in 20 parts of water at 80°, treated with decolorizing carbon, filtered and precipitated by adding first a few ml. of acetic acid and then hydrochloric acid to *p*H 2. A more crystalline product was obtained by initiating the precipitation with acetic acid. The yield was 85.2% based on anthranilic acid, m.p. 240–241° (dec.). Wheeler and Johns reported the melting point to be 230–232°. Purification was also effected by repeated crystallization of the sodium salt from twice its weight of water. Both products retained a slight color. Recrystallized from acetic acid it melted at 242.5–243.5° (dec.).

Anal. Calcd. for $C_7H_5O_2NI_2$: C, 21.60; H, 1.29; neut. equiv., 389. Found: C, 21.21, 21.56; H, 1.45, 1.62; neut. equiv., 387.

3-Amino-2,4,6-triiodobenzoic Acid (Procedure No. 2).—To a solution of *m*-aminobenzoic acid (68.5 g.) in water (2000 ml.) and hydrochloric acid (50 ml.) there was added a mixture of iodine monochloride (290 g.) and hydrochloric acid (290 ml.). Iodination was completed by heating at 80–85° with stirring for three hours. The yield of crude product was 236.3 g. (91.8%). It melted with decomposition at 190–192°. Purification was effected by crystallizing the sodium salt twice from 4–5 times its weight of water, dissolving at 60–70° and then cooling to 10° to crystallize. Liquors were used counter-currently. A final trace of color was removed most satisfactorily by dissolving the salt in ten times its weight of 1% sodium bisulfite, treating with decolorizing carbon at 60°, filtering, then heating to 80° and precipitating slowly by hydrochloric acid. The purified product weighed 190.8 g. (74.2%) and melted at 196.5–197.5° (dec.). Equivalent weight found 519; theory 515. The procedure described above is less time consuming than the original method of Kretzer[12] and gave a higher yield of purified product than the procedure of Goldberg and his associates.[4]

4-Amino-3,5-diiodobenzoic Acid (Procedure No. 3).—This product was prepared essentially according to the procedure of Michael and Norton.[13] The crude material was purified by crystallizing the sodium salt from 5% sodium chloride solution; yield of purified material 78.8%; neut. equiv., calcd. for $C_7H_5O_2NI_2$, 389; found, 381.

3-Acetylamino-2,4,6-triiodobenzoic Acid (Procedure No. 4).—3-Amino-2,4,6-triiodobenzoic acid (51.5 g.) was mixed with acetic anhydride (125 ml.) containing 2 drops of sulfuric acid and refluxed 30 minutes. After cooling, the mixture was stirred with 600 ml. of water until the excess acetic anhydride was hydrolyzed and crystallization was complete. The wet solid was suspended in 600 ml. of water and dissolved with a slight excess of ammonium hydroxide (1/2 hour). The solution was treated with decolorizing carbon, filtered and precipitated with an excess of hydrochloric acid (*p*H 1). The dried product weighed 51.5 g. (92.5% yield) and melted at 280–280.5° (dec.).

Anal. Calcd. for $C_9H_6O_3NI_3$: C, 19.40; H, 1.08; I, 68.50; neut. equiv., 557. Found: C, 19.77, 19.60; H, 1.59, 1.42; I, 68.55; neut. equiv., 565.

Mixed Anhydride of Acetic Acid and 3-Acetylamino-2,4,6-triiodobenzoic Acid.—Procedure No. 4 was repeated (using 26.0 g. of 3-amino-2,4,6-triiodobenzoic acid) except that acetic acid was fractionated off through a column during acetylation until the head temperature remained at 128° under total reflux. On cooling, the product crystallized and was washed with acetic anhydride; yield 26.8 g. melting at 227.5–228.5° (dec.). A further quantity (2.5 g.) was obtained by hydrolyzing the mother liquors; total yield 29.3 g. (98%). The compound was non-acidic but slowly dissolved in ammonium hydroxide to form a clear solution, indicating that under these conditions the amide of the iodinated acid was not formed.

(5) Dorothy R. Neuhaus, Adam A. Christman and Howard B. Lewis, *J. Lab. Clin. Med.*, **35**, 43 (1950).

(6) Reed M. Nesbit and Jack Lapides, University of Michigan Med. Bulletin XVI 37-42 (1950).

(7) UROKON is the trademark registered by the Mallinckrodt Chemical Works for 3-acetylamino-2,4,6-triiodobenzoic acid.

(8) Dorothy Neuhaus, Adam A. Christman and Howard B. Lewis, *Proc. Soc. Exp. Biol. Med.*, **78**, 313 (1951).

(9) Present address, Washington Univ., St. Louis, Mo.

(10) H. L. Wheeler and C. O. Johns, *Am. Chem. J.*, **43**, 405 (1910).

(11) C. J. Klemme and J. H. Hunter, *J. Org. Chem.*, **5**, 227 (1940).

(12) H. Kretzer, *Ber.*, **30**, 1944 (1897).

(13) A. Michael and L. M. Norton, *Am. Chem. J.*, **1**, 264 (1879-1880).

Anal. Calcd. for $C_{11}H_8O_4NI_3$: C, 22.04; H, 1.35. Found: C, 21.66, 21.74; H, 1.61, 1.42.

Anhydro 2-Acetylamino-3,5-diiodobenzoic Acid (Procedure No. 5).—2-Amino-3,5-diiodobenzoic acid (57.8 g.) was refluxed 30 minutes with 260 ml. of acetic anhydride containing 3 drops of sulfuric acid. Upon cooling, the anhydro form of 2-acetylamino-3,5-diiodobenzoic acid separated in large crystals; weight 58.5 g. (95% yield), m.p. 196–197.5°. Recrystallized from acetic acid, the product melted over a range from 197–214° but after crystallizing again from acetic anhydride it melted at 199–201° (dec.). It is probable that crystallization from acetic acid partially opens the ring of the inner anhydride. The material from acetic anhydride was analyzed.

Anal. Calcd. for $C_9H_5O_2NI_2$: C, 26.2; H, 1.20. Found: C, 26.35, 26.06; H, 1.50, 1.53.

2-(*n*-Caproylamino)-3,5-diiodobenzoic Acid (Procedure No. 6).—2-Amino-3,5-diiodobenzoic acid (9.7 g.) was mixed with 50 ml. of dioxane and 8 g. (8.4 ml.) of *n*-caproyl chloride and refluxed two hours. Hydrogen chloride ceased to be liberated after 1.5 hours. The mixture was then diluted with 50 ml. of water, warmed to 70–80° and sodium hydroxide solution was added, maintaining the mixture alkaline to phenolphthalein until all of the solid had dissolved (12 min.). The solution was diluted with 150 ml. of water, treated with decolorizing carbon, filtered again and precipitated hot (65–70°) from a volume of 6 l. by adding dilute hydrochloric acid to *p*H 4. The solid was filtered from the hot solution, washed and dried at 70°; weight 11.1 g. (90.5% of theory) melting at 205.5–206.5° (dec.).

Anal. Calcd. for $C_{13}H_{15}O_3NI_2$: C, 32.00; H, 3.08; neut. equiv., 487. Found: C, 32.30, 32.59; H, 3.19, 3.51; neut. equiv., 482.

2-Acetylamino-3,5-diiodobenzoic Acid (Procedure No. 7).—Ten grams of anhydro 2-acetylamino-3,5-diiodobenzoic acid was stirred with 50 ml. of dioxane and 50 ml. of water at room temperature and sodium hydroxide solution (35° Bé) was added drop by drop to maintain the mixture alkaline to phenolphthalein. The mixture consumed 3 ml. of the sodium hydroxide solution in 30 minutes. It was stirred an additional 10 minutes without *p*H change. The mixture was diluted with 100 ml. of water and made acid to litmus with acetic acid. No precipitate was formed, indicating the absence of unacetylated acid. The product was precipitated by hydrochloric acid, filtered, washed, and dried at 105°. It weighed 9.7 g. (93.4% yield) and melted at 225–227°. Recrystallized from acetic acid it melted at 228.5–230°.

Anal. Calcd. for $C_9H_7O_3NI_2$: C, 25.1; H, 1.62; neut. equiv., 430.8. Found: C, 25.14, 24.73; H, 1.81, 1.79; neut. equiv., 430.

3-Formylamino-2,4,6-triiodobenzoic Acid (Procedure No. 8).—3-Amino-2,4,6-triiodobenzoic acid (257.5 g., 0.5 mole) was suspended in 87% formic acid (1750 ml.) in a 5-l. flask fitted with stirrer, dropping funnel and thermometer. During 1.5 hours 1500 ml. of acetic anhydride was added, the mixture being well-stirred and maintained between 60 and 70°. A new and bulkier precipitate formed during the reaction. One thousand ml. of water was added slowly to decompose the excess acetic anhydride. The crude product, obtained by filtration, weighed 234 g. (86%). The product was purified by recrystallization from ethanol; over-all yield 71%. It melted at 252° (dec.). Analysis showed 69.0% iodine compared to the theoretical 70.5%. Repeated recrystallization failed to effect further purification.

3-Caproylamino-2,4,6-triiodobenzoic Acid (Procedure No. 9).—3-Amino-2,4,6-triiodobenzoic acid (103.0 g., 0.2 mole) was suspended in toluene (1100 ml.) in a 2-liter flask equipped with stirrer and condenser. Heat was applied and 250 ml. of toluene was distilled from the reaction mixture to remove any water. The condenser was set for reflux and 40 ml. (0.3 mole) of caproyl chloride was added. The reaction mixture was heated under reflux for one hour, cooled and the product separated by filtration, washed with ether and recrystallized from alcohol-water; over-all yield 65%. Calcd. neut. equiv., 613; found, 610.

ST. LOUIS 7, MISSOURI

[CONTRIBUTION FROM THE DEPARTMENTS OF RADIATION BIOLOGY AND BIOCHEMISTRY, THE UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE & DENTISTRY]

The Ozonolysis of Substituted Maleic Acid Imides and its Application to the Degradation of Porphyrins[1]

BY JONAS E. RICHMOND AND KURT I. ALTMAN

RECEIVED FEBRUARY 28, 1952

This paper describes a method for the decomposition of the oxidation products of protoporphyrin IX, by means of ozonolysis. The substituted maleic acid imides studied here form stable, crystalline ozonides which may be reductively decomposed, leading to identifiable α-keto acids.

In the course of investigations concerned with various phases of porphyrin metabolism using C^{14}-labeled precursors,[2] the need has arisen for a method for the degradation of the oxidative break-down products of such tetrapyrroles as protoporphyrin and chlorophyll. The oxidative degradation of porphyrins and related substances with chromic acid results in the formation of substituted maleic acid imides[3] whose further degradation is often desirable in order to determine the isotope concentration of individual carbon atoms in the porphyrin ring system. Although other approaches to this problem have been found feasible,[4] the method to be reported here[5] is accompanied by higher over-all yields.

The degradation of substituted maleic acid imides has been accomplished by formation of the corresponding stable ozonides with subsequent reductive decomposition of these ozonides according to the method described by Fischer and co-workers.[6] This approach is facilitated by the fact that the ozonides in question are crystalline solids which can be prepared in good yield. Owing to the relatively greater stability of the ozonides of substituted maleic acid imides, milder methods of decomposition were unsuccessful and the aforementioned

(1) This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

(2) K. I. Altman, G. W. Casarett, R. E. Masters, T. R. Noonan and K. Salomon, *J. Biol. Chem.*, **176**, 319 (1948); K. I. Altman, L. L. Müller and J. E. Richmond, *Arch. Biochem.*, **29**, 447 (1950); **36**, 399 (1952).

(3) H. Fischer and H. Orth, "Die Chemie des Pyrrols," Bd. II, 1. Hälfte, Akademische Verlagsgesellschaft m.b.H., Leipzig, 1937, p. 366.

(4) J. Wittenberg and D. Shemin, *J. Biol. Chem.*, **185**, 103 (1950).

(5) Preliminary Report: K. I. Altman and J. E. Richmond, *Abstr. XIIth Internat. Congr. Pure and Applied Chem.*, p. 70 (1951).

(6) F. G. Fischer, H. Düll and L. Ertel, *Ber.*, **65**, 1467 (1932).